

**This exam consists of 95 points on 6 pages. Allot 1 min/2 pts.**

1A. (4 pts) True & false (circle the correct answer).

T or **F**: All 20 amino acids contain at least one chiral center. [*Glycine has no chiral center*]

T or **F**: The peptide bond is planar and usually ~~is~~. [*Planer and trans*]

T or **F**: Non-polar residues are found in the core of globular proteins due to ~~van der Waals~~ forces. [*Hydrophobic*]

T or **F**: Disulfide bonds are usually found on ~~intra~~-cellular proteins. [*extra-cellular*]

T or **F**: If the ligand concentration is less than  $K_D$  then the fractional saturation is ~~greater than~~ 0.5. [*less than*]

T or **F**: Hydrogen bonds are ~~seldom~~ observed in protein-ligand interactions [*they can be common*]

T or **F**: Ligands that differ in their  $K_D$  values are more likely to have different kinetic ~~on-rates~~. [*off-rates differ*]

**T** or F: At equilibrium, the concentration of the protein-ligand complex is constantly changing, or fluctuating.

1B. Fill in the blanks (2 pts).

a. The antigen binding hypervariable loops are found on the variable domains of the heavy and light chains in antibodies.

b. An antibody can be cleaved to produce two (a number)  $F_{AB}$  fragments (1/2 pt).

2. (10 pts) Hydrogen Bonds:

i) Define/describe the general structure of a hydrogen bond. You answer should include a description of donor and acceptor groups (4 pts).

*X-H --- Y*

*Both X and Y are electronegative*

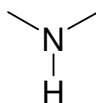
*X-H is the donor*

*Y is the acceptor*

*Partial positive charge on Hydrogen forms favorable electrostatic interaction with acceptor.*

ii) What chemical groups in a protein form main-chain hydrogen bonds? Illustrate your answer with a sketch. (2 pts).

*Between the N-H and the O=C*



iii) Briefly describe the importance of hydrogen bonds in stabilizing secondary structures of proteins (4 pts).

- Secondary structures form extensive mainchain hydrogen bonds.*
- Every residue (except those on the edge of sheets) forms an H-bond*
- Cause the enthalpy of denaturation to increase because a hydrogen bond in secondary structure is more stable than a hydrogen bond to water.*

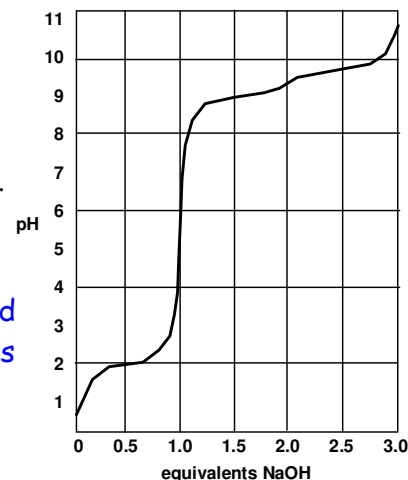
3. (5 pts) A titration curve for an amino acid is shown on the right.

i) What are the  $pK_a$  values for each ionization (1 pt)?

2, 9, ~9.5. There are three equivalents, therefore 3  $pK_a$  values.

ii) Briefly explain why weak acids act as buffers near their  $pK_a$  values (4 pts).

- In the buffer regions, the weak acid can ionize.
- The protons released by the weak acid neutralize the added base, therefore the hydrogen ion concentration decreases by a smaller amount = smaller changes in pH.



4. (10 pts) Please do **one** of the following two choices. Both choices utilize **0.1 M** acetate ( $pK_a=5.0$ ) as the buffer. Be sure to indicate the question that you are answering. Use the back of the previous page for calculations.

**Choice A:** Buffer construction, pH = 4, starting with NaAcetate.

- How many moles of NaAcetate would be needed to make 0.5 L of buffer? Please show your work (4 pts).
- If the desired pH of the buffer solution was 4.0, how many moles of HCl would have to be added to the solution of NaAcetate described in part i. Please show all of your work (6 pts).

**Choice B:** pH Adjustment, initial pH = 4, final pH = 6, restore to pH = 4.

- What is the total number of moles of acetic acid and/or acetate in 0.5 L of this buffer (at any pH value)? Please show your work (4 pts).
- The initial pH of your acetate buffered reaction was 4.0. The pH rises to 6.0 during the course of the reaction. How many moles of HCl do you have to add to restore the pH to 4.0? (6 pts).

For either question: # moles of acetic acid and/or acetate =  $[A_T] \times \text{vol} = 0.1 \times 0.5 = 0.05$  moles

**Choice A:** At pH 4.0,  $f_{HA} = 1/(1+R)$ .  $R = 10^{pH-pK_a} = 10^{4-5} = 10^{-1} = 0.1$ .  
 $f_{HA} = 1/1.1 = 0.91$

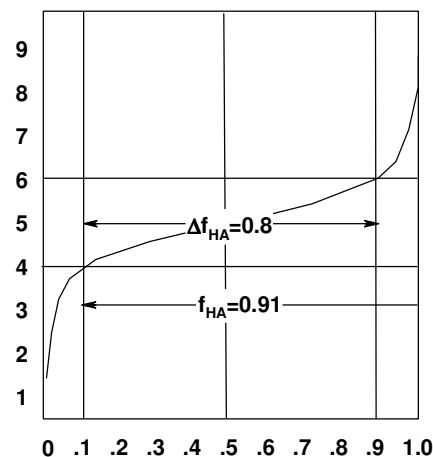
A total of  $0.91 \times .05 = 0.045$  moles of HCl

**Choice B:** At pH=4,  $f_{HA} = 0.9$ .

At pH=6,  $f_{HA} = 0.1$

Difference in fraction protonated is 0.8

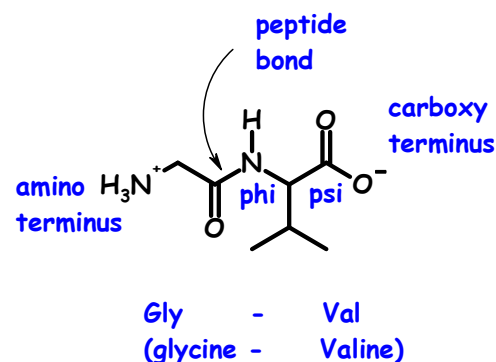
A total of  $0.8 \times .05 = 0.04$  moles of HCl would have to be added.



5. (10 pts) Draw the following dipeptide: Gly-Val, with the peptide bond in the *trans* conformation and in the correct ionization state for a pH of 6.0. If you do not know the structure of the sidechains for these amino acids, draw those that you do know, label them, and give the sequence of your modified peptide. Please do not use Glu, Phe, or Ile, as these are given elsewhere on the exam.

Label the following on your diagram:

- the amino terminus
- the carboxy terminus
- the peptide bond
- the single bonds corresponding to the phi and psi torsional angles (only one residue is necessary).



6. (12 pts) A protein contains three charged residues (A, B, C), the remaining residues are either polar or non-polar. The relative location of these three residues is shown in the diagram on the right, along with their  $pK_a$  values.

i) Write the names of residues A and C next to their structure (1 pt).

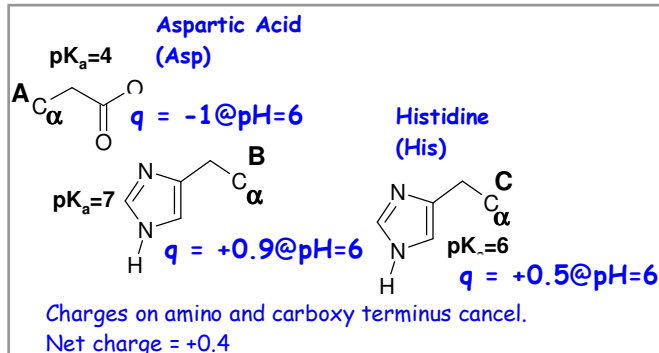
ii) Estimate the fraction protonated for each group, assuming a pH of 6.0. Use the back of the preceding page for calculations or sketching graphs. (3 pts).

**A:**  $f_{HA} = 0$ , since pH is 2 units above  $pK_a$ .

**B:**  $f_{HA} = 0.9$ , since pH is 1 unit below  $pK_a$ .

**C:**  $f_{HA} = 0.5$  since pH is equal to  $pK_a$ .

iii) Estimate the net charge on this protein at pH=6.0, don't forget to include contributions from the amino terminus and the carboxy terminus. Use the back of the previous page if you need additional space. (4 pts)



$$pH = pK_a + \log \frac{[A^-]}{[HA]}$$

$$R = 10^{(pH - pK_a)}$$

$$f_{HA} = \frac{1}{1 + R} \quad f_{A^-} = \frac{R}{1 + R}$$

$q_{TOTAL} = \sum f_{HA} q_{HA} + f_{A^-} q_{A^-}$	$f_{HA}$	$q_{HA}$	$f_{HA} \times q_{HA}$	$f_{A^-}$	$q_{A^-}$	$f_{A^-} \times q_{A^-}$
Amino Term ( $pK_a=9$ )	1.0	+1	+1	0	0	0
Carboxy term, ( $pK_a=2$ )	0	0	0	1.0	-1	-1
A (Asp), $pK_a=4$	0	0	0	1.0	-1	-1
B (His), $pK_a=7$	0.9	+1	+0.9	0.1	0	0
C (His), $pK_a=6$	0.5	+1	+0.5	0.5	0	0
<b>The overall charge is +2.4 -2.0 = +0.4.</b>			+2.4			-2.0

Instead of doing the entire table, you also have used the following approach.

- the amino terminus will have a +1 charge at this pH since it is fully protonated; the carboxy will be fully deprotonated with a charge of -1, so there is no contribution from the termini.
  - The Asp residue is fully deprotonated ( $pH \gg pK_a$ ) and has a neg. charge:  $q = -1.0$
  - His (B) is + when protonated, the fraction protonation = 0.9 (pH one less  $pK_a$ ):  $q = +0.9$
  - His (C) is + when protonated, the fraction protonation = 0.5 ( $pH = pK_a$ ):  $q = +0.5$
- SUM: = +0.4**

iv) \*\*Explain why the two chemically identical side chains have different  $pK_a$  values, assume the group with the  $pK_a=6.0$  has the same  $pK_a$  as the free amino acid (2 pts). Use the back of the previous page to answer this question.

The negative charge on the Aspartic acid residue will stabilize, or lower the energy of the  $HA^+$  form of histidine B due to a favorable electrostatic interaction. This will make the group a weaker acid, which explains the  $pK_a$  shift from 6 to 7.

v) Where would you typically find residues of this type: on the surface, or in the core (circle choice)? (2 pts).

7. (8 pts) Please do **one** of the following **two** choices, the second choice is found on the following page.

**Choice A:** A peptide was digested with Chymotrypsin. The peptides from this digest were separated and the sequence of the first five residues of each peptide were determined using Edman degradation, giving the following result:

Ala-Asp-Asp-Phe

Ser-Gly-Met-Lys-Val

A new sample of the peptide was cleaved with cyanogen bromide, and the first five residues of each peptide were:

Ala-Asp-Asp-Phe-Ser

Lys-Val-Leu-Ser

A new sample of the same peptide was cleaved with trypsin, and the first five residues of each peptide were:

Ala-Asp-Asp-Phe-Ser

Val-Leu-Ser

What is the complete amino acid sequence of the peptide (you should check your answer to verify that it would account for the above data)?

**The sequence is:** Ala-Asp-Asp-Phe-Ser-Gly-Met-Lys-Val-Leu-Ser

**Chymotrypsin:** Ala-Asp-Asp-Phe Ser-Gly-Met-Lys-Val-Leu-Ser

**Cyanogen bromide:** Ala-Asp-Asp-Phe-Ser-Gly-Met Lys-Val-Leu-Ser

**Trypsin:** Ala-Asp-Asp-Phe-Ser-Gly-Met-Lys Val-Leu-Ser

**Choice B:** A protein does not contain tyrosine or phenylalanine. A 1  $\mu\text{M}$  ( $10^{-6}$  M) solution of this protein has a UV absorbance of 0.02. How many tryptophan residues are present in this protein?

$$A = \epsilon[X]l, \quad l = 1\text{ cm}$$

$$\epsilon_{\text{TRP}} = 5,000 \text{ M}^{-1}\text{cm}^{-1}$$

You need to determine the molar extinction coefficient first, and then determine the number of Trp residues:

$$\epsilon = A/[X] = 0.02/1 \times 10^{-6} = 2 \times 10^4$$

$$\epsilon = n_{\text{TRP}} \times \epsilon_{\text{TRP}}$$

$$n = 2 \times 10^4 / 5,000 = 4 \text{ Tryptophan residues.}$$

8. (6 pts) Please do one of the following choices:

**Choice A:** Sketch an  $\alpha$ -helix, indicate the location of a few hydrogen bonds and sidechains in your sketch. Also indicate the number of residues/turn.

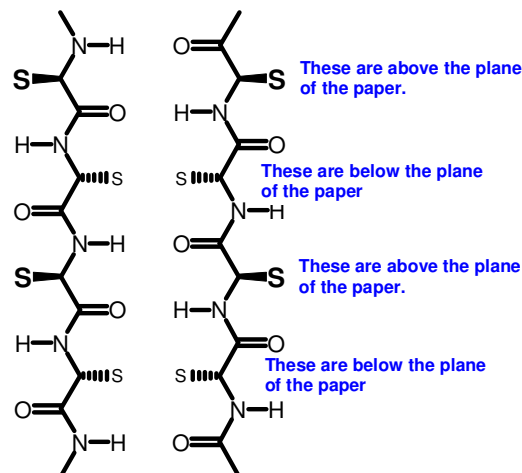
Helix-like drawing (1 pt)

H-bonds should be parallel to helix axis (2 pts)

Sidechains should point out from the helix (2 pts)

3.6 residues/turn (1 pt)

**Choice B:** Is the  $\beta$ -sheet shown on the right parallel or anti-parallel? Briefly justify your answer. Describe, or draw, the orientation of the sidechains in this sheet.



The sheet is anti-parallel (+3 pts). The left-most strand runs from N  $\rightarrow$  C from top to bottom. The right-most strand runs from N  $\rightarrow$  C from bottom to top (1 pt).

The sidechains point up and down, i.e. every second residue points up (2 pts).

**Choice C:** Pick any super-secondary structure. Describe, or sketch, its structure and briefly discuss the intramolecular forces that stabilize it.

$\beta$ - $\alpha$ - $\beta$  an alpha helix placed on top of a two stranded  $\beta$ -sheet (2 pts)

H-bonds would stabilize the individual secondary structures. (2 pts)

The sheet would have a non-polar face, which would contact the non-polar face of the helix.

The helix-sheet interaction is stabilized by the hydrophobic effect and van der Waals (2 pts)

$\beta$ -barrel:  $\beta$ -strands arranged in a barrel (3 pts), stabilized by mainchain hydrogen bonds (2pts) and to some extent van der Waals interactions between the sidechains (1 pt).

9. (12 pts) There are two *entropy* terms that are important in protein folding/unfolding. Briefly describe **both** of these two terms and indicate how they stabilize (or destabilize) the native, or folded form of a protein.

i) Conformational entropy (+2 pts). The large number of conformations of the unfolded chain (2 pts) destabilizes the native form since the increase in entropy during unfolding is favorable (2 pts).

ii) Hydrophobic effect (2 pts): Exposure of buried non-polar side chains to the solvent decreases the order of the water (2 pts). This stabilizes the folded form since a decrease in entropy of the solvent due to unfolding is unfavorable (2 pts).

10. (12 pts) Please do one of the following two choices:

**Choice A:** A 8 residue segment of a protein is found on the *surface* of a protein. The sequence of this segment is:

-Glu-Phe-Glu-Phe-Glu-Phe-Glu-Phe-

The Ramachandran plot for this segment of the protein is shown to the right. Each “dot” represents the phi and psi angles for a residue in this sequence.

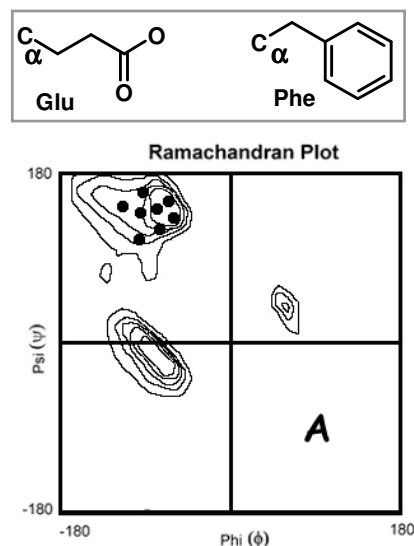
i) What is the most likely secondary structure for this section of the protein?

Why would it be energetically favorable for this segment to form this structure? Briefly justify your answer. (8 pts)

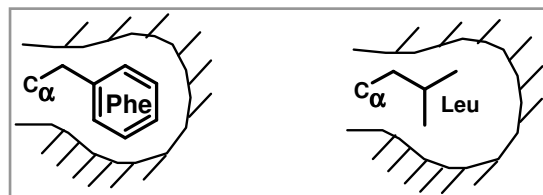
- The Ramachandran plot indicates that the structure is a  $\beta$ -strand. An alternative explanation is that the alternating polar (Glu) non-polar (Phe) suggest a b-strand (3 pts).
- This secondary structure would be polar on one side (Glu) and non-polar on the other surface. The non-polar surface would face the core of the protein and the polar surface would be exposed to the solvent (4 pts).
- This would be energetically favorable because the non-polar Phe residues would be buried (hydrophobic effect.) (1 pts)

ii) Why is it energetically unfavorable to find residues in the region labeled “A” (4 pts)

These phi & psi angles will generate unfavorable van der Waals contacts (repulsive forces) between the sidechain and main chain atoms.



**Choice B:** A protein normally contains a phenylalanine residue buried in its non-polar core (left structure). Would replacement of this residue by leucine (right structure) increase or decrease the following terms? Briefly justify your answer. Assume the reaction direction is *native*  $\rightarrow$  *unfolded*.

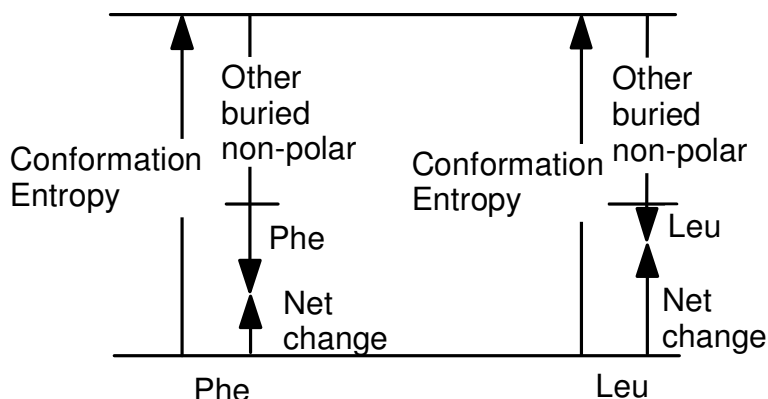


i) The  $\Delta H^\circ$  of unfolding.

The  $\Delta H^\circ$  of unfolding would decrease (but still be positive) due to loss of van der Waals interactions since the Ile is smaller than Phe (+6 pts).

ii) The  $\Delta S^\circ$  of unfolding.

The overall  $\Delta S^\circ$  of unfolding would increase. Leu is smaller than Phe and will therefore show a smaller decrease in entropy due to the hydrophobic effect (6 pts, diagram not necessary).

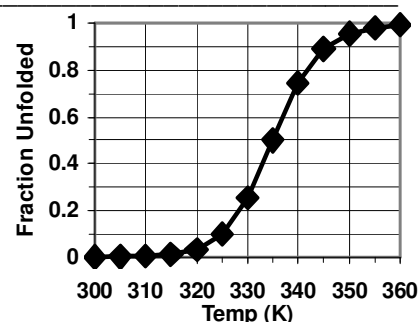


11. (4 pts) Do **one** of the following choices. Indicate your choice.

**Choice A:** The curve to the right shows a denaturation curve for a protein.

- What is the standard energy change,  $\Delta G^\circ$  at  $T = 335\text{ K}$  (2 pts)?
- Determine the equilibrium constant at  $T = 335\text{ K}$ ? (2 pts).

Since  $335\text{ K}$  is  $T_M$ , where  $f_N = f_U$ ,  $\Delta G^\circ = 0$  and  $K_{EQ} = 1$ .



**Choice B:** A protein denatures with a  $\Delta H^\circ$  of  $+200\text{ kJ/mol}$  and an entropy change,  $\Delta S^\circ$  of  $+600\text{ J/mol-K}$ .

- Calculate the equilibrium constant and fraction folded (native, N) at  $300\text{ K}$ .  $R = 8.31\text{ J/mol-K}$  (4 pts)

$$\Delta G^\circ = \Delta H^\circ - T\Delta S^\circ = -RT \ln K_{EQ}$$

$$f_N = \frac{1}{1 + K_{EQ}} \quad f_U = \frac{K_{EQ}}{1 + K_{EQ}}$$

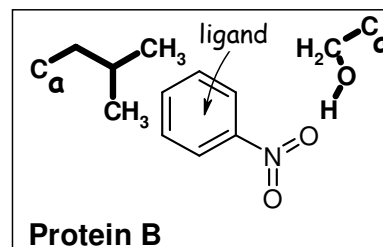
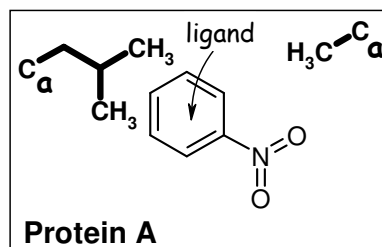
$$\Delta G^\circ = +200,000 - (300)(600) = +20,000$$

$$K_{EQ} = e^{-20,000 / (8.31 \times 300)} = 3.2 \times 10^{-4}$$

$$f_N = 1$$

**Choice C:** Two different proteins bind the same ligand, nitrobenzene. The structure of the protein-ligand complexes are shown on the right. The two sidechains from the protein that contact the ligand are in bold.

Which protein would show a *lower*  $K_D$ , protein A or protein B? Why?



Protein B has more interactions (the hydrogen bond to the nitro group) so it will have the smaller  $K_D$ .

Average = 84 %

